

CLAIMS

What is claimed is:

1. A method for treating a disease or medical disorder in a mammal, comprising administering to the mammal a nitrosating agent.
2. The method of Claim 1 in which the nitrosating agent is selected for rapid entry into a target cell.
3. The method of Claim 1, wherein the disease or medical disorder is selected from the group consisting of: shock, angina, stroke, reperfusion injury, acute lung injury, sickle cell anemia and infection of red blood cells.
4. A composition comprising SNO-Hb[FeII]O<sub>2</sub> which is S-nitrosylated without detectable oxidation of the heme Fe.
5. A composition comprising SNO-Hb[FeII] which is S-nitrosylated without detectable oxidation of the heme Fe.
6. A method for delivering NO in a mammal, comprising administering to the mammal a blood substitute comprising nitrosated hemoglobin.
7. The method of Claim 6, in which the blood substitute further comprises low molecular weight S-nitrosothiol.
8. A method for treating a disease or medical condition in a mammal, comprising administering to the mammal a composition comprising a form of nitrosated or

5 nitrated hemoglobin, wherein the disease or medical condition is selected from the group consisting of heart disease, brain disease, vascular disease, atherosclerosis, lung disease, inflammation, stroke, angina and respiratory distress.

9. A method for treating a human with sickle cell anemia comprising administering to the human a composition comprising  $\text{SNO-Hb(FeII)O}_2$ .

10. The method of Claim 9 in which the composition further comprises a thiol.

11. The method of Claim 9 in which the composition further comprises an S-nitrosothiol.

12. A method for treating a patient having a disease or medical condition characterized by abnormalities of nitric oxide and oxygen metabolism, comprising administering to the patient an effective amount of a preparation comprising nitrosated hemoglobin.

13. The method of Claim 12 in which the disease or medical condition is selected from the group consisting of: heart disease, lung disease, sickle-cell anemia, stroke, sepsis and organ transplantation.

14. A blood substitute comprising nitrosated or nitrated hemoglobin.

15. A method for treating a disorder resulting from platelet activation or adherence in an animal or human, comprising administering to the animal or human a composition comprising nitrosated or nitrated hemoglobin in a therapeutically effective amount.

16. The method of Claim 15 wherein the disorder is selected from the group consisting of: myocardial infarction, pulmonary thromboembolism, cerebral thromboembolism, thrombophlebitis, sepsis and unstable angina.

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*Sub B1*  
17. A method for preventing thrombus formation in an animal or human, comprising administering a composition comprising nitrosated hemoglobin in a therapeutically effective amount.

10 18. A method for forming polynitrosated hemoglobin, comprising combining hemoglobin with an excess of S-nitrosothiol over hemoglobin tetramer in an aqueous solution, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on hemoglobin.

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19. A method for forming polynitrosated or polynitrated hemoglobin in which heme Fe is in the FeII state, comprising combining hemoglobin with an NO donating compound, maintaining the resulting combination under conditions appropriate for nitrosation or nitration to occur, thereby forming polynitrosated or polynitrated hemoglobin, and reacting the polynitrosated or polynitrated hemoglobin with a reagent which selectively reduces FeIII to FeII.

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20. The method of Claim 19 in which the reagent which selectively reduces FeIII to FeII is a cyanoborohydride.

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21. The method of Claim 19 in which the reagent which selectively reduces FeIII to FeII is methemoglobin reductase.

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22. A composition comprising polynitrosated hemoglobin.
23. Method for treating or preventing a disease or medical disorder which can be ameliorated by delivery of NO or its biological equivalent to tissues affected by the disease or medical disorder, in an animal or human, comprising administering to the animal or human nitrosyl-heme-containing donors of NO.
24. The method of Claim 23 wherein the nitrosyl-heme-containing donor of NO is nitrosylhemoglobin.
25. Method for making stable nitrosyl-deoxyhemoglobin comprising adding NO to deoxyhemoglobin in an aqueous solution such that the ratio of NO:heme is less than about 1:100 or greater than about 0.75.
26. Method for making SNO-oxyhemoglobin, comprising adding NO to an aqueous solution of oxyhemoglobin and buffer having a pK of at least about 9.4, at a concentration of approximately 10 mM to 200 mM, at pH 7.4.
27. Method for making SNO-oxyhemoglobin, comprising adding NO to an aqueous solution of oxyhemoglobin in approximately 10 mM phosphate buffer at pH 7.4.
28. A composition comprising nitrosyl-deoxyhemoglobin in a physiologically compatible buffer, wherein the ratio of NO:heme is less than about 1:100 or greater than about 0.75.
29. Method for making nitrosyl-oxyhemoglobin comprising adding NO to oxyhemoglobin in an aqueous solution such that the ratio of NO:hemoglobin is less than about 1:30.

30. Hemoglobin conjugated to an NO-donor.

31. Hemoglobin of Claim 30, wherein the NO-donor is selected from the group consisting of:  
diazoniumdiolates, nitroprusside, nitroglycerin and nitrosothiol.

32. A composition comprising hemoglobin and one or more NO-donors.

33. A method for treating or preventing a disease or medical disorder which can be ameliorated by delivery of NO or its biological equivalent to tissues affected by the disease or medical disorder, in an animal or human, comprising administering a heme-based blood substitute and inhaled NO to the animal or human.

34. A method for delivering CO to the tissues in an animal or human, comprising administering CO-derivatized hemoglobin to the animal or human.

35. A method of treating or preventing a disease or medical disorder which can be ameliorated by delivery of NO or its biological equivalent to tissues affected by the disease or medical disorder in an animal or human, comprising administering both CO-derivatized hemoglobin and a nitrosated hemoglobin to the animal or human.

36. Nitrosylhemoglobin conjugated to one or more electron acceptors.

37. Nitrosylhemoglobin of Claim 36, wherein the electron acceptor is selected from the group consisting of superoxide dismutase, stable nitroxide radicals,

oxidized forms of nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide, flavin mononucleotide, ascorbate and dehydroascorbate.

- 5 38. A composition comprising nitrosylhemoglobin and one or more electron acceptors.
39. A composition comprising S-nitrosohemoglobin in a pharmaceutically acceptable vehicle, wherein the S-nitrosohemoglobin is selected from the group  
10 consisting of SNO-Hb[FeII]O<sub>2</sub>, SNO-Hb[FeII]CO, and SNO-Hb[FeIII].
40. A method for measuring nitrosyl(FeII)-hemoglobin in blood comprising the steps of:  
15 a) lysing the red blood cells of a blood sample;  
b) preparing a protein fraction of the lysed red blood cells;  
c) subjecting a protein fraction to photolysis; and  
d) determining the amount of nitric oxide in the protein fraction by measuring a chemiluminescence  
20 signal generated by a chemical reaction between nitric oxide and ozone.
41. A method for measuring nitrosyl(FeII)-hemoglobin in venous blood comprising the steps of:  
25 a) lysing the red blood cells in a sample of venous blood;  
b) isolating a desalted protein fraction of the lysed red blood cells;  
c) subjecting the desalted protein fraction to photolysis; and  
30 d) determining the amount of nitric oxide in the desalted protein fraction by measuring a

chemiluminescence signal generated by a chemical reaction between nitric oxide and ozone.

42. A method for assaying S-nitrosohemoglobin comprising:
- a) isolating washed red blood cells from blood;
  - 5 b) lysing the red blood cells, thereby obtaining a lysate;
  - c) desalting the lysate;
  - d) contacting an aliquot of the lysate of c) with mercury ions in excess over protein
  - 10 concentration, thereby obtaining a mercury-treated aliquot and an untreated aliquot;
  - e) exposing the mercury-treated aliquot and the untreated aliquot to oxygen;
  - f) measuring NO equivalents in the mercury-treated
  - 15 aliquot of e) and NO equivalents in the untreated aliquot of e) by photolysis-chemiluminescence; and
  - g) determining a quantity of S-nitrosohemoglobin from the NO equivalents measured in f).
- 20 43. A method for assaying S-nitrosohemoglobin comprising:
- a) isolating washed red blood cells from blood;
  - b) lysing the red blood cells, thereby obtaining a lysate;
  - c) desalting the lysate;
  - 25 d) contacting an aliquot of the lysate with mercury ions in excess over protein concentration, thereby obtaining a mercury-treated aliquot and an untreated aliquot;
  - e) exposing the mercury-treated aliquot and
  - 30 untreated aliquot to oxygen;
  - f) isolating a mercury-treated low molecular weight fraction and an untreated low molecular weight fraction from the respective aliquots of e);

- g) contacting the low molecular weight fractions of f) with excess low molecular weight thiol under acidic conditions, thereby producing S-nitrosothiol in each fraction;
- 5 h) measuring NO equivalents from S-nitrosothiol in the fractions of g) by photolysis-chemiluminescence; and
- i) determining a quantity of S-nitrosohemoglobin from the NO equivalents measured in h).
- 10 44. A method for measuring S-nitrosohemoglobin and nitrosyl(FeII)-hemoglobin in red blood cells, comprising:
- a) isolating washed red blood cells from blood;
- 15 b) lysing the red blood cells, thereby obtaining a lysate;
- c) desalting the lysate; and
- d) measuring NO equivalents from the lysate of c) by photolysis-chemiluminescence.
- 20 45. A method for measuring nitrosyl(FeII)-hemoglobin in venous blood, comprising:
- a) isolating washed red blood cells from venous blood;
- b) lysing the red blood cells, thereby obtaining a lysate;
- 25 c) desalting the lysate; and
- f) determining NO equivalents in the lysate by photolysis-chemiluminescence, thereby measuring nitrosyl(FeII)-hemoglobin.
- 30 46. A method for assaying nitrosyl(Fe)-hemoglobin comprising:
- a) isolating washed red blood cells from blood;



- 5      b) lysing the red blood cells, thereby obtaining a lysate;  
c) desalting the lysate;  
d) contacting an aliquot of the lysate with mercury  
ions in excess over protein concentration,  
thereby obtaining a mercury-treated aliquot and  
an untreated aliquot;  
e) exposing the mercury-treated aliquot and  
untreated aliquot to oxygen;  
10      f) measuring NO equivalents in the aliquots of e) by  
photolysis-chemiluminescence, thereby obtaining  
1) a quantity for S-nitrosohemoglobin and 2) a  
quantity for nitrosyl(FeII)-hemoglobin and S-  
nitrosohemoglobin combined, respectively; and  
15      g) determining a quantity for nitrosyl(FeII)-  
hemoglobin by subtracting 1) from 2).

47. A method for assessing oxygen delivery to a site in  
the body, comprising determining S-nitrosohemoglobin  
and nitrosylhemoglobin values in a blood sample from  
20 the site, and using said values to assess oxygen  
delivery.

48. The method of Claim 47, further comprising determining  
an oxygen value in the blood sample and using said  
value to assess oxygen delivery.

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a1

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B1